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## Original article

## A prospective study of travellers' diarrhoea: analysis of pathogen findings by destination in various (sub)tropical regions

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## ABSTRACT

**Objectives:** Eighty million travellers visiting (sub)tropical regions contract travellers' diarrhoea (TD) each year, yet prospective data comparing the prevalence of TD pathogens in various geographical regions are scarce. Our recent study using modern molecular methods found enteropathogenic (EPEC) and enterotoxigenic (EAEC) *Escherichia coli* to be the most frequent pathogens, followed by enterotoxigenic *E. coli* (ETEC) and *Campylobacter*. We revisited our data to compare the findings by geographical region. **Methods:** A total of 459 prospectively recruited travellers provided stool samples and completed questionnaires before and after visiting destinations in various geographical regions. A multiplex quantitative real-time PCR assay was used to analyse *Salmonella*, *Yersinia*, *Campylobacter jejuni*/*Campylobacter coli*, *Shigella*, *Vibrio cholerae*, EPEC, EAEC, ETEC, enterohaemorrhagic *E. coli* and enteroinvasive *E. coli*.

**Results:** TD was contracted by 69% (316/459) of the subjects; EPEC and EAEC outnumbered ETEC and *Campylobacter* in all regions. Multiple pathogens were detected in 42% (133/316) of the samples. The proportions of all pathogens varied by region. The greatest differences were seen for *Campylobacter*: while relatively frequent in South Asia ( $n = 11$ ; 20% of the 55 with TD during travel) and Southeast Asia (15/84, 15%), it was less common in East and West Africa (5/71, 7% and 1/57, 2%) and absent in South America and the Caribbean (0/40).

**Conclusions:** EPEC and EAEC outnumbered ETEC and *Campylobacter* everywhere, yet the proportions of pathogen findings varied by region, with ETEC and *Campylobacter* rates showing the greatest differences. The high frequency of multibacterial findings in many regions indicates a need for further investigation of the clinical role of each pathogen. **T. Lääveri, Clin Microbiol Infect 2018;24:908.e9–908.e16**

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## Introduction

Travellers' diarrhoea (TD) accounts for more than 80 million cases each year [1]. It is not only the disease most commonly encountered by travellers to (sub)tropical destinations [2,3] but also the most common health complaint at posttravel consultations [2,4]. Bacterial pathogens are known to predominate as aetiological agents. However, the pathogen remains unidentified in up to half of cases [5,6]. Over the past decade the field of TD research has experienced a renaissance, with new molecular methods covering a larger variety of pathogens than traditional approaches. However,

few data are available comparing the incidence of the various pathogens by geographical area.

Until recently, enterotoxigenic *Escherichia coli* (ETEC) has been reported to outnumber all other pathogens causing TD in most regions [5,6], with the exception of Southeast Asia (Thailand), where *Campylobacter* has been considered the primary pathogen [5–9]. Studies using modern molecular methods have decreased the proportion of unexplained TD cases to only 4% to 24% [10–12]. These investigations have also revealed diarrhoeagenic *E. coli* (DEC) to be even more prevalent than previously thought. The rates for enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (EAEC) have been at least as high as those for ETEC even after visiting Central America [11,13], South Asia [10] or Africa [10,12,14], where earlier findings have been predominated by ETEC. Other bacterial pathogens such as *Shigella*,

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*Salmonella* spp., enteroinvasive and enterohaemorrhagic *E. coli* (EIEC and EHEC respectively), *Aeromonas* spp., *Plesiomonas shigelloides*, *Arcobacter* spp., enterotoxigenic *Bacteroides fragilis* and *Vibrio* spp. have been detected less frequently [5,6].

We found only a handful of reports providing data on TD pathogens by region in a single study using modern molecular methods. EPEC has only been covered in a few investigations. Moreover, EAEC prevalence data among travellers to Southeast Asia and East Africa remain virtually nonexistent.

In our prospective study of 459 Finnish travellers, we applied multiplex quantitative real-time PCR (qPCR) methodology to analysis of various TD pathogens by region, seeking to challenge the current understanding of regionally predominant pathogens.

## Materials and methods

### Study population and recruitment

The participants for this prospective study were initially enrolled at the Travel Clinic of Aava Medical Centre, Helsinki, Finland, over a 12-month period in 2009–2010 before their journey outside the Nordic countries for more than four nights. Of the 526 volunteers, 459 (63%) completed questionnaires and provided stool samples before and after travel (Supplementary Fig. S1). Ethical approval was obtained from the ethics committee of the department of medicine at Helsinki University Hospital. All study subjects provided written informed consent. We earlier reported the findings of resistant *Enterobacteriaceae* [15] and travel-related health problems [3] in the same volunteers. Recently we described the stool pathogens of 382 travellers who did not use antimicrobials [16].

### Definition of TD

TD was defined according to World Health Organization criteria as passage of loose or liquid stools more frequently than is normal for the individual (<http://www.who.int/topics/diarrhoea/en/>). Severe TD was defined as six or more diarrhoeal stools per day, TD accompanied by fever or haemorrhagic stools, or TD requiring hospitalization.

### Questionnaires

The pretravel questionnaire included demographic data and information on possible diarrhoeal symptoms at the time of the first faecal sample. The posttravel questionnaire assessed the travel itinerary, diarrhoeal and other symptoms, and medications during or immediately after travel.

### Travel destinations

The countries visited were grouped into regions as modified from the United Nations classification: South Asia; Southeast Asia; East Asia; North Africa and Middle East; East Africa; West Africa (Western and Middle Africa); Southern Africa; Latin America (South and Central America and the Caribbean); and Europe, Australia and North America. The 37 travellers (8%) who visited more than one region were grouped by longest stay; 16 (43%) of these visited Europe or the United States on their way to the (sub)tropics. This report focuses on the five most popular destinations: South Asia, Southeast Asia, East Africa, West Africa and Latin America.

### Collection and laboratory analysis of stool samples

Briefly, stool samples were collected before departure and from the first (or second) stool passed after returning home as swabs in

Copan M40 Transystem tubes (Copan Diagnostics, Brescia, Italy) and mailed in special boxes, reaching the laboratory within 1 to 3 days. Once the samples arrived, total nucleic acids were extracted using the standard semiautomated protocol of easyMAG (bio-Mérieux, Marcy l'Etoile, France). The analyses were carried out with a multiplex qPCR method [1] which covers the following pathogens: *Campylobacter jejuni* and *Campylobacter coli*, *Salmonella* spp., *Yersinia*, *Vibrio cholerae*, EPEC, EAEC, ETEC, EHEC and EIEC/*Shigella* spp. Here we focus on the findings of pathogens found to associate with ongoing TD symptoms discussed in our previous report (EPEC, EAEC, ETEC and *Campylobacter*) [16].

### Statistical analysis

Statistical analyses were carried out by SPSS 22 (IBM SPSS, Chicago, IL, USA).

The chi-square test or binary logistic regression analysis was used to compare categorical variables when applicable. A binomial regression model was used to obtain profile likelihood confidence intervals (CIs) for the proportions of different pathogens in geographical regions; in cases when computation did not converge, asymptotic Wald CIs were used. Statistical significance was determined as either 95% CIs not overlapping, or ranging only either above or below 1.

## Results

### Demographic data and occurrence of TD

Background data on the travellers and their travels are shown in Supplementary Table S1. TD was reported by 316 travellers (69%). At the time of the posttravel stool sample, 143 (31%) reported having ongoing symptoms, 173 (38%) reported that symptoms had already resolved and 143 (31%) were asymptomatic through the entire journey. Seventy-two (16%) reported having taken antimicrobial medications during travel. TD was most common among those who visited South Asia (55/69, 80%), Southeast Asia (84/108, 79%) and East Africa (71/96, 74%), while 67% (57/85) of those who travelled to West Africa and 60% (24/40) to Latin America reported TD (Supplementary Table S2). There were no significant differences between regions in the severity of TD (severe vs. nonsevere TD, *p* 0.118; data not shown).

### Pathogen findings in stool samples of all 459 travellers

Nineteen (4%) of 459 pretravel samples were positive for bacterial pathogens; only one subject with EPEC reported mild diarrhoeal symptoms. An analysis of the posttravel stool samples of all 459 subjects (Table 1) revealed EPEC (*n* = 194; 42%) and EAEC (192; 42%) to be the most common pathogens, outnumbering both ETEC (88; 19%) and *Campylobacter* (31; 7%). EHEC was identified in 37 (8%), *Salmonella* in 11 (2%) and EIEC/*Shigella* in six (1%) stool samples. *Vibrio cholerae* and *Yersinia* were not detected. Two or more types of bacterial pathogens were found in 168 (37%) of all post-travel faecal samples; 57 (12%) had three or more pathogens.

### EPEC, EAEC, ETEC and *Campylobacter* findings in stool samples of travellers to five regions

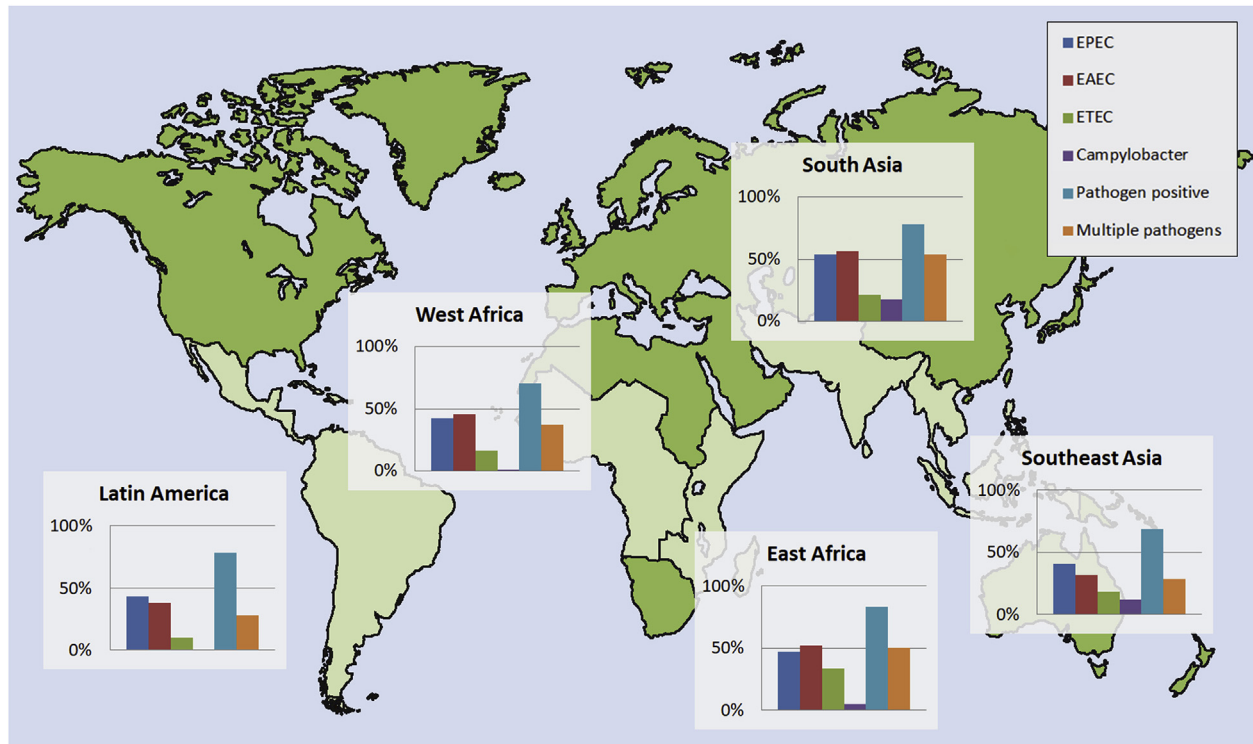
The findings of EPEC, EAEC, ETEC and *Campylobacter* in relation to symptoms among travellers to South Asia, Southeast Asia, East Africa, West Africa and Latin America are presented in Supplementary Table S2 and in Figs. 1–3. Pathogen findings from countries with more than ten visitors are presented in Supplementary Table S3.

**Table 1**

Bacterial pathogens detected in posttravel stool samples of 459 Finnish travellers in relation to symptoms and geographical region

Characteristic	All travellers	Any pathogen	Multiple pathogens	EPEC	EAEC	ETEC	EHEC	Only DEC	Only non-DEC	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Shigella</i> /EIEC
Total (% of all)	459	326 (71)	168 (37)	193 (42)	192 (42)	88 (19)	37 (8)	223 (49)	12 (3)	31 (7)	11 (2)	6 (1)
Proportion of travellers with TD by pathogen finding												
TD during travel	316 (69)	241 (74)	133 (79)	134 (69)	152 (79)	76 (86)	27 (73)	159 (71) <sup>a</sup>	11 (92) <sup>a</sup>	30 (97)	8 (73)	5 (83)
OR (95% CI) TD during travel vs. no TD <sup>a</sup>		2.2 (1.4–3.3)	2.2 (1.4–3.5)	1.0 (0.7–1.6)	2.4 (1.6–3.7)	3.5 (1.8–6.6)	1.2 (0.6–2.6)	1.6 (1.0–2.4)	8.5 (1.1–67.8)	14.9 (2.0–110.3)	1.2 (0.3–4.6)	2.3 (0.3–19.7)
Findings by geographical region												
Asia (all)	184 (40)	130 (71)	69 (38)	83 (45)	74 (40)	35 (19)	10 (5)	78 (42)	12 (7)	25 (14)	7 (4)	2 (1)
South Asia	69 (15)	54 (78)	37 (54)	37 (54)	39 (57)	15 (22)	7 (10)	29 (42)	2 (3)	12 (17)	1 (1)	2 (3)
Southeast Asia	108 (24)	74 (68)	31 (29)	44 (41)	34 (31)	20 (19)	3 (3)	48 (44)	10 (9)	13 (12)	6 (6)	0 (0)
East Asia	7 (2)	2 (29)	1 (14)	2 (29)	1 (14)	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Africa (all)	218 (47)	162 (74)	86 (39)	93 (43)	100 (46)	48 (22)	19 (9)	116 (53)	0 (0)	6 (3)	3 (1)	4 (2)
North Africa and Middle East	13 (3)	8 (62)	1 (8)	5 (39)	3 (23)	0 (0)	1 (8)	7 (54)	0 (0)	0 (0)	0 (0)	0 (0)
Southern Africa	24 (5)	14 (58)	5 (21)	7 (29)	8 (33)	2 (8)	2 (8)	11 (46)	0 (0)	0 (0)	0 (0)	0 (0)
West (and Middle) Africa	85 (19)	60 (71)	32 (38)	36 (42)	39 (46)	14 (16)	7 (8)	40 (47)	0 (0)	1 (1)	1 (1)	4 (5)
East Africa	96 (21)	80 (83)	48 (50)	45 (47)	50 (52)	32 (33)	9 (9)	58 (60)	0 (0)	5 (5)	2 (2)	0 (0)
Latin America (South and Central America and Caribbean)	40 (9)	31 (78)	11 (38)	17 (43)	15 (38)	4 (10)	7 (18)	26 (65)	0 (0)	0 (0)	1 (3)	0 (0)
Europe, Australia, North America	17 (4)	3 (18)	2 (12)	0 (0)	3 (18)	1 (6)	1 (6)	3 (18)	0 (0)	0 (0)	0 (0)	0 (0)

Data are presented as *n* (%) unless otherwise indicated. No *Yersinia* or *Vibrio cholerae* were detected.CI, confidence interval; DEC, diarrhoeagenic *Escherichia coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; OR, odds ratio; TD, travellers' diarrhoea.<sup>a</sup> Compared to no bacterial pathogens.

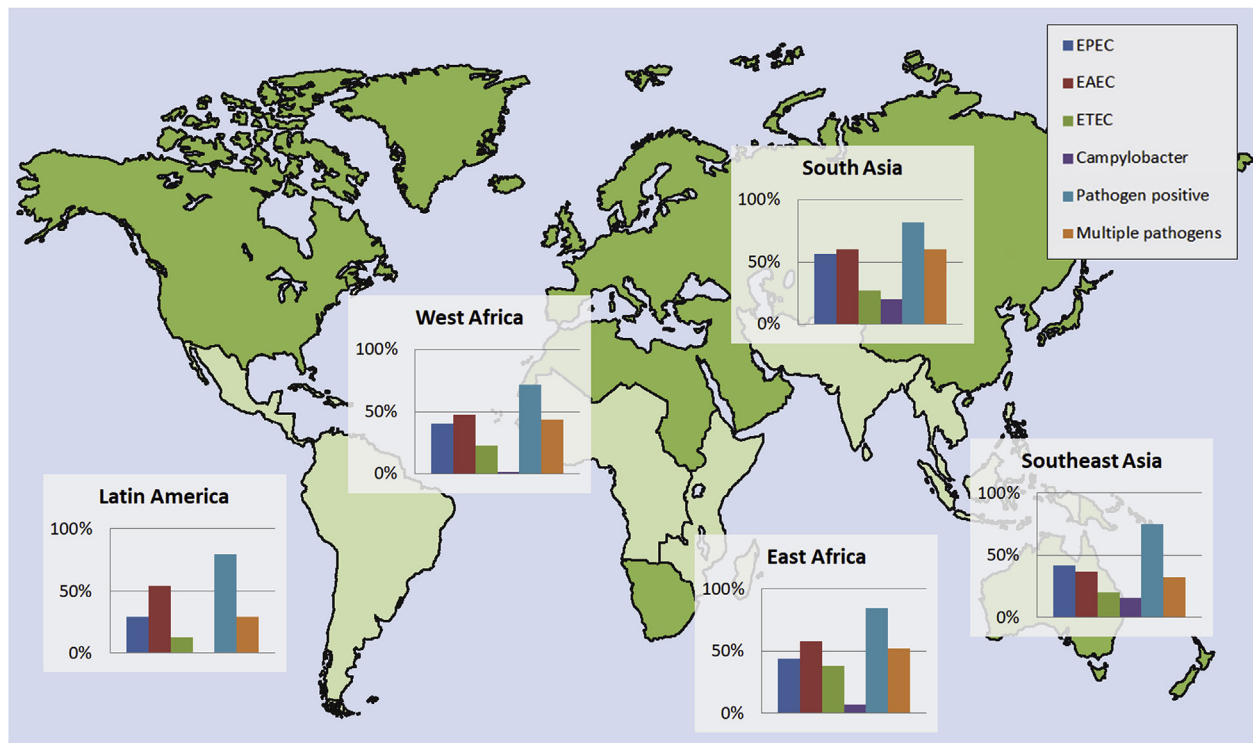


**Fig. 1.** Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among 459 Finnish travellers to South Asia, Southeast Asia, East Africa, West Africa and Latin America. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*.

#### TD pathogens of travellers to South Asia

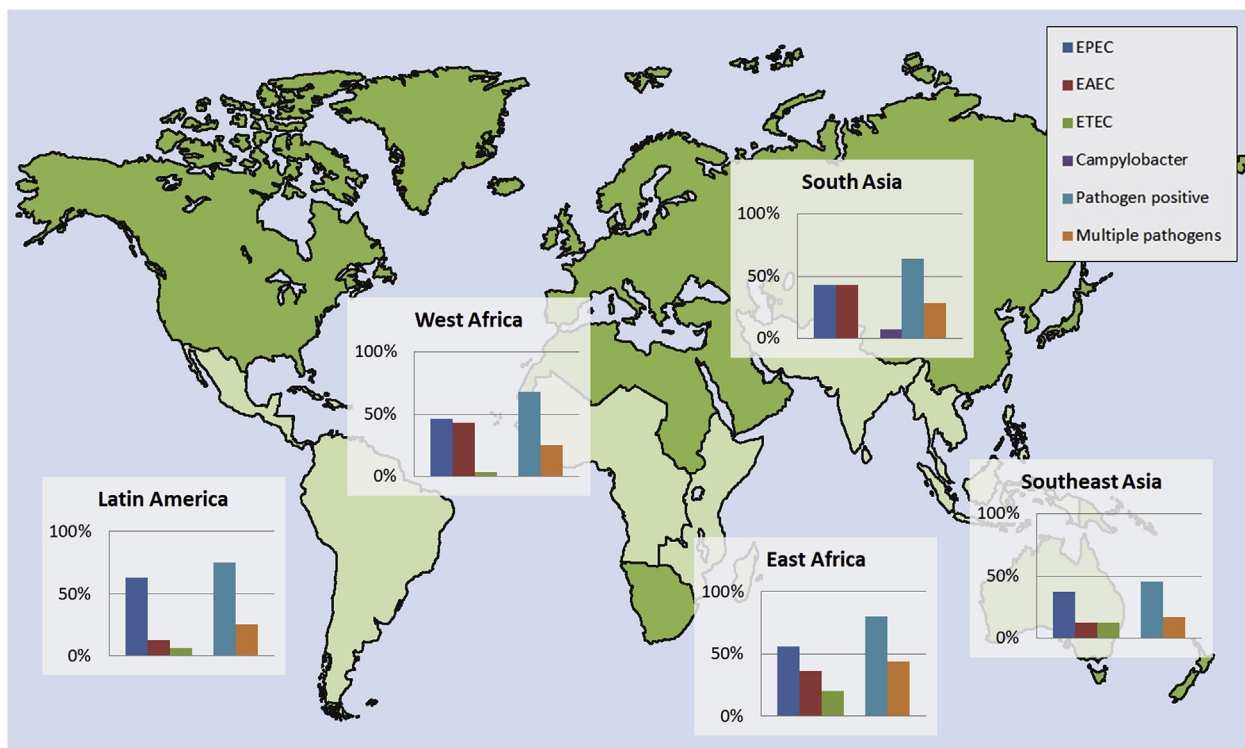
Bacterial pathogens were found in 82% (45/55) of stool samples from travellers to South Asia with TD. EAEC (33/55, 60%) and EPEC (31/55, 56%) were more common findings than ETEC (15/55, 27%)

and *Campylobacter* (11/55, 20%); 60% (33/55) had two or more types of pathogens and 31% (14/55) had three or more. Of those whose symptoms had already resolved, *Campylobacter* was found in 30% (8/27). Of asymptomatic travellers, 64% (9/14) had a pathogen finding; 43% (6/14) had either EAEC or EPEC.



**Fig. 2.** Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among travellers with TD during travel. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; TD, travellers' diarrhoea.





**Fig. 3.** Map showing proportions of findings for EPEC, EAEC, ETEC and *Campylobacter*, any pathogen or multiple pathogens among travellers who remained asymptomatic during travel. EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*.

#### TD pathogens of travellers to South Asia

Among the 108 travellers to Southeast Asia, pathogens were found in 75% (63/84) of those with TD. EPEC (35/84, 42%) and EAEC (31/84, 37%) were the most frequent findings, followed by ETEC (17/84, 20%) and *Campylobacter* (13/84, 15%). Among travellers with ongoing TD, multiple pathogens were detected in 50% (12/24), EPEC in 50% (12/24), EAEC in 38% (9/24), ETEC in 33% (8/24) and *Campylobacter* in 17% (4/24). Of those remaining asymptomatic, 46% (11/24) had a pathogen finding.

#### TD pathogens of travellers to West Africa

Among the 85 travellers to West Africa, pathogens were found in 72% (41/57) of those with TD. EAEC was detected in 47% (27/57) and EPEC in 40% (23/57), followed by ETEC in 23% (13/57) and *Campylobacter* in 2% (1/57). Two or more types of pathogens were found in 44% (25/57). In asymptomatic travellers, 68% (19/28) had pathogen findings.

#### TD pathogens of travellers to East Africa

Of the 96 travellers to East Africa, a pathogen was detected in the faecal samples of 85% (60/71) of those with TD. ETEC was almost as common (27/71, 38%) as EPEC (41/71, 58%) and EAEC (41/71, 44%). *Campylobacter* was detected in 7% (5/71). Two or more different types of pathogens were detected in 52% (37/71). These proportions were similar among travellers with ongoing TD. Of asymptomatic travellers, 80% (20/25) had pathogen findings.

#### TD pathogens of travellers to Latin America

Among 40 travellers to Latin America, pathogens were found in 79% (19/24) of those having experienced TD. EAEC (14/24, 54%) and

EPEC (7/24, 30%) were found more commonly than ETEC (3/24, 13%); 30% (7/24) had two or more types of pathogens. *Campylobacter* was not detected. Among travellers with ongoing TD, EAEC, EPEC and ETEC were found in equal proportions (3/10, 30%). Of asymptomatic travellers, 75% (12/16) had pathogen findings.

#### Differences between regions by pathogens found in travellers with TD during travel

Geographical comparisons by pathogen of travellers with TD are presented in Table 2. The results with statistical significance are provided here.

##### EPEC

The region with the highest proportion was South Asia (56%); the comparison to Latin America was significant (29%, odds ratio (OR) 0.3, 95% CI 0.1–0.9).

##### EAEC

This pathogen was found more frequently in samples from travellers to South Asia than Southeast Asia (60% vs. 37%, OR 0.4, 95% CI 0.2–0.8).

##### ETEC

The highest proportion of this pathogen was detected among those visiting East Africa (38%); the comparison to Southeast Asia (20%, OR 0.4; 95% CI 0.2–0.9) and Latin America (13%, OR 0.2; 95% CI 0.1–0.9) was significant.

##### *Campylobacter*

This pathogen was most commonly detected among travellers to South Asia (20%); the proportions were significantly lower in West Africa (2%, OR 0.1, 95% CI 0.01–0.6) and East Africa (7%, OR 0.3, 95% CI 0.1–0.9). No cases with *Campylobacter* were detected in Latin America.

**Table 2**

Relationship among pathogen findings and travel destination among travellers with TD during travel

Pathogen and travel destination	All travellers			TD during travel			Ongoing TD			Asymptomatic		
	n (%) of travellers to region	p	OR (95% CI)	n (%) of travellers to region	p	OR (95% CI)	n (%) of travellers to region	p	OR (95% CI)	n (%) of travellers to region	p	OR (95% CI)
Any pathogen												
South Asia	54 (79)	0.412	0.7 (0.3–1.6)	45 (82)	0.688	0.8 (0.3–2.1)	24 (86)		1.0	9 (64)	0.286	0.5 (0.1–2.0)
Southeast Asia	74 (69)	0.015	0.4 (0.2–0.9)	63 (75)	0.148	0.6 (0.2–1.2)	18 (75)	0.334	0.5 (0.1–2.0)	11 (46)	0.016	0.2 (0.1–0.8)
West Africa	60 (71)	0.043	0.5 (0.2–1.0)	41 (72)	0.087	0.5 (0.2–1.1)	19 (73)	0.256	0.5 (0.1–1.8)	19 (68)	0.320	0.5 (0.2–1.9)
East Africa	80 (83)		1.0	60 (85)		1.0	33 (85)	0.901	0.9 (0.2–3.6)	20 (80)		1.0
Latin America	31 (78)	0.425	0.7 (0.3–1.7)	19 (79)	0.547	0.7 (0.2–2.3)	7 (70)	0.281	0.4 (0.1–2.2)	12 (75)	0.706	0.8 (0.2–3.4)
Multiple pathogens												
South Asia	37 (54)		1.0	33 (60)		1.0	18 (64)		1.0	4 (29)	0.346	0.5 (0.1–2.1)
Southeast Asia	31 (29)	0.001	0.3 (0.2–0.7)	27 (32)	0.001	0.3 (0.2–0.6)	12 (50)	0.300	0.6 (0.2–1.7)	4 (17)	0.044	0.3 (0.1–1.0)
West Africa	32 (38)	0.048	0.5 (0.3–1.0)	25 (44)	0.089	0.5 (0.2–1.1)	13 (50)	0.291	0.6 (0.2–1.7)	7 (25)	0.149	0.4 (0.1–1.4)
East Africa	48 (50)	0.646	0.9 (0.5–1.6)	37 (52)	0.141	0.7 (0.4–1.5)	18 (46)	0.145	0.5 (0.2–1.3)	11 (44)		1.0
Latin America	11 (28)	0.009	0.3 (0.1–0.8)	7 (29)	0.014	0.3 (0.1–0.8)	3 (30)	0.071	0.2 (0.1–1.1)	4 (25)	0.223	0.4 (0.1–1.7)
EPEC												
South Asia	37 (54)		1.0	31 (56)		1.0	17 (61)		1.0	6 (43)	0.285	0.5 (0.1–1.9)
Southeast Asia	44 (41)	0.094	0.6 (0.3–1.1)	35 (42)	0.091	0.6 (0.3–1.1)	12 (50)	0.439	0.6 (0.2–1.9)	9 (38)	0.125	0.4 (0.1–1.3)
West Africa	36 (42)	0.165	0.6 (0.3–1.2)	23 (40)	0.091	0.5 (0.2–1.1)	14 (54)	0.610	0.8 (0.3–2.2)	13 (46)	0.307	0.5 (0.1–1.8)
East Africa	45 (47)	0.393	0.8 (0.4–1.4)	31 (44)	0.158	0.6 (0.3–1.2)	18 (46)	0.241	0.6 (0.2–1.5)	14 (56)	0.681	0.8 (0.2–2.8)
Latin America	17 (43)	0.264	0.6 (0.3–1.4)	7 (29)	0.029	0.3 (0.1–0.9)	3 (30)	0.105	0.3 (0.1–1.3)	10 (63)		1.0
EAEC												
South Asia	39 (57)		1.0	33 (60)		1.0	18 (64)		1.0	6 (43)		1.0
Southeast Asia	34 (31)	0.001	0.4 (0.2–0.7)	31 (37)	0.008	0.4 (0.2–0.8)	9 (38)	0.057	0.3 (0.1–1.0)	3 (13)	0.043	0.2 (0.04–1.0)
West Africa	39 (46)	0.190	0.7 (0.3–1.2)	27 (47)	0.181	0.6 (0.3–1.3)	12 (46)	0.183	0.5 (0.2–1.4)	12 (43)	1.000	1.0 (0.3–3.7)
East Africa	50 (52)	0.573	0.8 (0.4–1.6)	41 (56)	0.799	0.9 (0.4–1.9)	19 (49)	0.208	0.5 (0.2–1.4)	9 (36)	0.673	0.8 (0.2–2.9)
Latin America	15 (38)	0.057	0.5 (0.2–1.0)	13 (54)	0.629	0.8 (0.3–2.1)	3 (30)	0.071	0.2 (0.1–1.1)	2 (13)	0.074	0.2 (0.03–1.2)
ETEC												
South Asia	15 (22)	0.106	0.6 (0.3–1.1)	15 (27)	0.206	0.6 (0.3–1.3)	10 (36)	0.660	0.8 (0.3–2.2)	0 (0)	NA	
Southeast Asia	20 (19)	0.017	0.5 (0.2–0.9)	17 (20)	0.016	0.4 (0.2–0.8)	8 (33)	0.542	0.7 (0.2–2.1)	3 (13)	0.481	0.6 (0.1–2.7)
West Africa	14 (16)	0.011	0.4 (0.2–0.8)	13 (23)	0.067	0.5 (0.2–1.1)	7 (27)	0.247	(0.5 80.2–1.6)	1 (4)	0.092	0.1 (0.02–1.4)
East Africa	32 (33)		1.0	27 (38)		1.0	44 (35)		1.0	5 (20)		1.0
Latin America	4 (10)	0.008	0.2 (0.1–0.7)	3 (13)	0.028	0.2 (0.1–0.9)	3 (30)	0.526	0.6 (0.1–2.7)	1 (6)	0.249	0.3 (0.03–2.5)
Campylobacter												
South Asia	12 (17)		1.0	11 (20)		1.0	3 (11)	0.534	0.6 (0.1–3.0)	1 (7)	NA	NA
Southeast Asia	13 (12)	0.321	0.7 (0.3–1.5)	13 (15)	0.491	0.7 (0.3–1.8)	4 (17)		1.0	0 (0)	NA	NA
West Africa	1 (1)	0.006	0.1 (0.01–0.4)	1 (2)	0.013	0.1 (0.01–0.6)	0 (0)	0.998	NA	0 (0)	NA	NA
East Africa	5 (5)	0.016	0.3 (0.1–0.8)	5 (7)	0.037	0.3 (0.1–0.9)	3 (8)	0.282	0.4 (0.1–2.1)	0 (0)	NA	NA
Latin America	0 (0)	0.998	NA	0 (0)	0.998	NA	0 (0)	0.999	NA	0 (0)	NA	NA

CI, confidence interval; EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; OR, odds ratio; TD, travellers' diarrhoea.

### Multiple pathogen findings

Multiple pathogen findings were more frequent among travellers to South Asia (60%) than Southeast Asia (32%; OR 0.3, 95% CI 0.2–0.6) or Latin America (29%; OR 0.3, 95% CI 0.1–0.8).

## Discussion

The perception of TD aetiology has been revolutionized by modern molecular methods offering high sensitivity and covering a wide range of pathogens. ETEC has long been considered the most common TD pathogen (apart from in Thailand) [5,6]. Our earlier data showed EPEC and EAEC to predominate [16], which is in accord with other recent findings [10–12,14]. The regional differences revealed by our analysis were mostly related to ETEC and *Campylobacter*. We next discuss our pathogen findings by region.

### South Asia: multiple pathogen findings

Our data show multiple pathogens to be especially common among travellers to South Asia. Of those with ongoing TD, 64% had more than one pathogen in their stool samples. This corresponds with earlier investigations reporting rates of 27% to 60% for multiple pathogens [17,18]. We also detected pathogens in the stools of 74% of the asymptomatic travellers, 29% of whom were coinfecting. Indeed, our data indicate that travellers visiting the region are very commonly exposed to stool pathogens.

Some reports suggest that *Campylobacter* may be contracted in South Asia as frequently as in Southeast Asia [18–20]. Correspondingly, we found *Campylobacter* among 20% and 15% of travellers with TD visiting South Asia and Southeast Asia, respectively. However, even in South Asia, EAEC (60%) and EPEC (56%) were the most common pathogens, followed by ETEC (27%). Although consistent with some other studies [10,17], this observation is contradicted by Jiang et al. [18], who reported only 5% to be infected with EAEC, but 76% with ETEC and 20% with *Campylobacter*. The varying detection rates for EAEC may be explained by methodologic differences. In our data, DEC strains were seen in all but two stool samples with a pathogen finding.

### Southeast Asia: DEC more common than *Campylobacter*

Review articles looking at travellers to Southeast Asia report *Campylobacter* to be the most common TD pathogen (25–35%), with low proportions of DEC infections [5,6]. Our findings in this geographical region contradict previous reports. We detected both EPEC and EAEC (42% and 37% of travellers with TD, respectively) more frequently than *Campylobacter* (15%); ETEC was seen in 20%. One reason for overestimating the role of *Campylobacter* may be the limited coverage of the various DEC in earlier investigations among travellers to Southeast Asia; we are not aware of any studies covering all four pathogens—EAEC, EPEC, ETEC and *Campylobacter*—in a single report. Moreover, it should be noted that most research into TD in Thailand has been conducted by studying military personnel instead of ordinary tourists [7–9]. The high rate of DEC (87% of our pathogen findings) in our data shows a greater prevalence of these pathogens in Southeast Asia than previously reported.

### West Africa: DEC predominate

The few fairly recent studies of TD aetiology in Western or Middle Africa [10,12,14] have presented results concurring with ours showing EAEC and EPEC (47% and 40% of those with TD during travel) to be at least as common as ETEC (23%) among travellers to West Africa. These data dispute earlier investigations finding ETEC

predominant and other DEC uncommon [21–23]. Together with the very low rates of *Campylobacter* (2%), our data indicate that DEC also predominates in this geographical area.

### East Africa: ETEC especially common

Interestingly, in East Africa, coinfections were almost as common as in South Asia; DEC were identified in all stool samples with a positive pathogen finding. We are aware of only two studies published over the past 30 years analysing TD among travellers to East Africa. Among 464 travellers, Jiang et al. [24] identified ETEC in the specimens of 35% visiting Kenya (5% had *Campylobacter*; EPEC or EAEC were not screened for), and Paschke et al. [10] reported that of 12 travellers to East Africa, six had EAEC, four had heat-labile ETEC and four heat-stable ETEC; EPEC was not analysed. In our data, East Africa was the region with the highest rate of ETEC, which corresponds with the rates in earlier reports [10,24], yet here also EPEC and EAEC were equally frequent. Our five *Campylobacter* cases (7% of those 71 with TD) in East Africa and one case (1/57, 2%) in West Africa support earlier observations [19] of a tenfold difference in the risk between East and West Africa. The cause of the great regional variations in *Campylobacter* findings among travellers has remained unclear.

### Latin America: other DEC as common as ETEC

Our data accord with recent studies [11,13,17,25] indicating considerable frequency of EAEC in Latin America [18,26], but contradict others reporting EAEC in only 3% to 13% of TD cases [18,26]. To our knowledge, only one study has included EPEC in the analyses of TD pathogens in Latin America [11]; our results showing 29% of travellers with TD to have EPEC concur with these findings. In our data, EPEC and EAEC were as commonly found as ETEC. Consistent with earlier investigations, we found no cases of *Campylobacter* [5,6,27]. It should be noted that only seven of our 40 volunteers visited Mexico or Guatemala, where the earlier aetiological studies have mostly been conducted. Nevertheless, EPEC and EAEC should evidently also be included in future analyses of the aetiology of TD in Latin America.

### Study limitations

Multiplex PCR methods have been criticized as having too high a sensitivity, as they may identify pathogens in too scanty a number to cause clinical symptoms. At the same time, it has been recognized that besides the quantity of pathogens, the development of symptoms also depends on a number of other factors, such as the infective dose and the host's preexisting immunity. Furthermore, multiplex PCR assays, by covering a wide selection of pathogens, reveal the multibacterial nature of TD diseases, thus suggesting that older studies with narrower coverage of pathogens may have misinterpreted the role and prevalence of individual findings. The role of each pathogen needs to be studied further. As a limitation, our multiplex qPCR did not cover any viral or parasitic pathogens.

Because the stool samples were taken only after travel, the prevalence of some pathogens may have been underestimated. EPEC and ETEC were more likely to be found in the specimens of travellers with ongoing symptoms than those recovered from the disease, whereas equal rates of *Campylobacter* and EAEC were detected in both groups [16]. Indeed, at least ETEC has been suggested to disappear rapidly from stools [25]. Such characteristics of these pathogens may lead to underestimating the rates of ETEC and EPEC, thereby overemphasizing the role of *Campylobacter* and EAEC. Moreover, despite being more common among travellers with TD than those asymptomatic in our earlier report [16], the role of EPEC in TD remains disputable.



## Practical considerations

Our data indicate that in all the geographical regions we selected for study, DEC are found in the majority of TD cases, with EPEC and EAEC as predominating findings. However, it should be pointed out that the clinical picture of the disease caused by different pathogens may vary. *Campylobacter* has been associated with more severe TD including fever and abdominal pain [28,29] and ETEC with acute watery diarrhoea [29]. One study suggests similar clinical pictures for EPEC and ETEC [28]. Although we failed in our previous report to find differences in the severity of TD caused by the various TD pathogens, EPEC and EAEC were also commonly detected in the stool samples of asymptomatic travellers, while ETEC and *Campylobacter* were almost always associated with symptomatic disease [16]. Therefore, the prevalence rate of a pathogen should not be equated to its clinical pertinence.

## Conclusions

When using modern molecular methods with enhanced coverage of bacterial pathogens, fewer stools remain without any findings (in our data 26% of those with TD during travel). DEC were revealed to be the most prevalent pathogens in all regions encompassed by the study. Indeed, while the proportions of ETEC and *Campylobacter* were similar or slightly lower than in earlier reports, the main benefit of modern techniques lies in revealing the concomitant high prevalence of EPEC and EAEC. Regional differences were mostly seen in the prevalence of ETEC and *Campylobacter*, yet the multibacterial nature of the findings attests to the need to redefine the clinical picture and prevalence of each pathogen.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.cmi.2017.10.034>.

## Transparency Declaration

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